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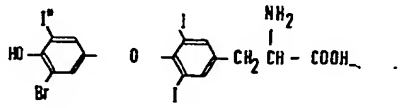
(54) Radioactive Carboxylic Acid Derivatives and Processes for Their Manufacture

(57) There is provided radioactive carboxylic acid derivatives containing I^{125} or I^{131} suitable for use in the quantitative determination of thyroxine or its metabolites in a patients blood by radioimmuno assay methods.

The preferred derivative has the structure formula



(3'Br,5'I*, 3,5-diodothyronine)

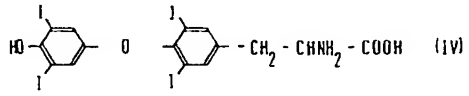
A method for the preparation of the radioactive carboxylic acid derivative is given.

SPECIFICATION

Radioactive Carboxylic Acid Derivatives and Processes for Their Manufacture

The present invention relates to radioactive carboxylic acid derivatives, a process for their manufacture and their use in radioimmuno assays.

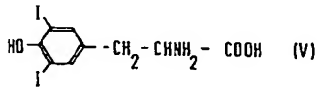
Thyroxine (T_4) is a thyroid hormone having the following formula:



(IV)

In order to recognise the occurence of disturbed thyroid functions or to follow the course of a treatment therefore, it is necessary to quantitatively determine this hormone and its metabolites, e.g. triiodothyronine or tetraiodothyroacetic acid, in the patient's blood. At present the thyroxine concentration is usually determined by radioimmuno-assay, which requires a thyroxine antibody and radioactively labelled thyroxine. The thyroxine antibody is obtained by immunising experimental animals with the acid of a T_4-protein conjugate and subsequent collection of blood serum from these animals.

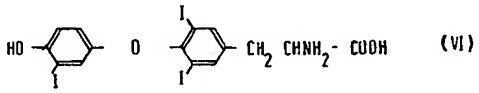
Thyroxine metabolites, e.g. diiodotyrosine which has the structure:



(V)

can be determined by analogous methods.

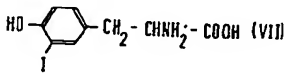
Radioactively labelled thyroxine can be obtained by the method of Hunter (as modified by J. Weeke and H. Orskov, Scand, J. Clin. Lab. Invest., 1973, *32* 357) by reacting 3,3',5-triiodothyronine (T_3)



(VI)

with radioactive iodine and chloramin T (the sodium salt of N-chloro-4-methylbenzenesulphonamide).

Corresponding radioactive labelled thyroxine metabolites can be obtained by analogous methods. For instance, diiodotyrosine can be obtained from iodotyrosine



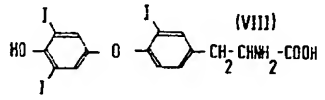
(VII)

by this method.

Radioimmuno-assay involves incubating the serum sample to be analysed for thyroxine with radioactively labelled thyroxine and with the thyroxine antibody, the latter being used in an amount less than theoretical as compared with the amount of thyroxine present in the sample together with the added radioactively labelled thyroxine. The thyroxine present in the serum sample competes with the radioactively labelled thyroxine for bonding positions on the thyroxine antibody. The resultant antigen-antibody complex is then separated from the unbound hormone, for instance by precipitation, following which radioactivity is measured in one of the two phases. The amount of thyroxine present in the sample to be analysed can then be determined with the acid of a calibration curve.

Radioactively labelled thyroxine is also required for determining thyroxine by other methods, e.g. the competitive protein binding assay (CPBA) and for the determination of other parameters, e.g. thyroxine-binding globulins.

The amount of other thyroxine metabolites formed in the body e.g. 3,3',5'-triiodo-L-thyronine (T_3):



(VIII)

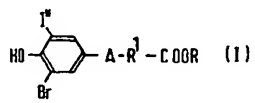
(VIII)

the concentration of which in the serum or in other body fluids enables other pathological conditions to be investigated, may be determined by analogous proceedures.

It has been found, however, that in the preparation of the above-mentioned tracer compounds labelled with radioactive iodine, by iodination of the appropriate carboxylic acid derivatives (e.g. using the method of Hunter, employing radioactive iodine and chloramine T), the substitution reaction is accompanied by exchange of an iodine substituent already present on the same benzene ring. It is therefore possible to obtain, for instance, radioactively labelled thyroxine under the above iodination conditions by iodine exchange from thyroxine itself. As a result of this exchange reaction it is found that in the preparation of thyroxine labelled in the outer ring, increasing amounts of doubly labelled molecules are formed since a second radioactive iodine-substituent is introduced in some of the molecules. Such doubly labelled compounds are, however, undesirable since they decompose more rapidly.

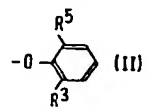
This invention provides radioactive carboxylic acid derivatives in which the above-mentioned disadvantage of double labelling does not occur.

Accordingly, therefore, the present invention provides radioactive carboxylic acid derivatives having the following general formula



(I)

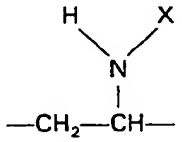
where A represents a direct bond or a group having the general formula



(II)

in which the ether oxygen is bound directly to the benzene ring in formula (I); R is a hydrogen atom

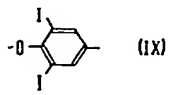
or a lower alkyl group; R^1 is a direct bond or one of the following groups namely: $—CH_2—$, $—CH_2—CH_2—$ or



(where X=H a lower alkyl group or a lower alkanoyl group); and R^3 and R^5 may each be H, Br or I, independently of each other.

The terms 'lower alkyl group' and 'lower alkanoyl group' as used in the present specification and in the claims refer to alkyl or alkanoly groups containing 1 to 4 carbon atoms. Examples of lower alkyl groups are methyl, ethyl, propyl, isopropyl, n-butyl, sec.-butyl, and tert. butyl groups. Examples of lower alkanoyl groups are formyl, acetyl and propionyl groups.

In view of the great importance of the determination of thyroxine in serum, the radioactive compound according to the invention having the formula I, where A represents a group having the formula

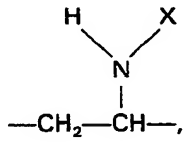


(IX)

R^1 is an aminoethylene group and R=H, plays a role of special importance.

The radioactive iodine present in the compounds according to the invention may be ^{125}I or ^{131}I. ^{125}I is preferred for radioimmuno-assays.

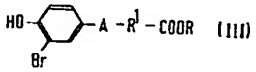
Compounds according to the invention, in which R^1=



may equally well be used in the D-, L or DL- form, though the L-form is preferred, since the compounds formed by the organism are likewise present in the L-form.

It has been established that the compounds according to the present invention posses practically the same reactivity towards the corresponding antibodies as those compounds which contain an iodine atom instead of a bromine atom. For instance an 85—100% crossed reactivity between 3'-bromo-5'(iodo(^{125}I))-3,5-diiodo-L-thyronine and a thyroxine antiserum. The reactivity of an antibody towards substances other than the one against which it was produced is referred to as 'crossed reactivity' and is expressed as a percentage, taking the reactivity with respect to the corresponding antigen as equal to 100%.

Since practically no exchange reaction, i.e. no double labelling (<1%) occurs during the preparation of the compounds according to the invention, on account of the greater stability of the bromine-carbon bond, as compared with the iodine-carbon bond, the compounds according to the invention are distinguished by greater stability and applicability as compared with conventional tracers, especially at high specific activities (the theoretically possible specific activity can be attained). It is thus possible when carrying out radioimmuno-assays to achieve an increased sensitivity without the disadvantage of the shorter working life of conventional tracers having a high specific activity. The compounds according to the invention can be obtained by reacting a compound having the general formula



(III)

where the symbols have the same significance as before, with a radioactive iodine and with chloramin T in a known manner.

The invention is explained in more detail by means of the following examples.

Example 1

3'-Bromo-5'-(iodo(^{125}I))-3,5-diiodo-L-thyronine

0.4 μg of 3'-bromo-3,5-diiodo-L-thyronine in 10 μl of buffer solution (0.5 M phosphate, pH 7.5) was added to 0.5 mCi of sodium iodide (^{125}I, no carrier) in 10 μl of the same buffer solution. The mixture was then stirred well and 9 μg of chloramin T in 10 μg of the same buffer solution was added. After 30 sec. the reaction was stopped by addition of 25 μg of sodium metabisulphite in 10 μl of water. The resultant product was purified by column chromatography. For this purpose it is charged into a column of cross-linked dextran gel (e.g. Sephadex G-25), on which unreacted iodide is first eluted with 0.05 M tris (hydroxymethyl) aminomethane/hydrochloric acid buffer (pH 9). Elution with this buffer is then continued, with addition of methanol. This results in the elution of the desired product.

Alternatively, the product can be subjected to layer chromatography on paper or silica gel.

The starting material employed in the above process can be obtained as follows:

0.51 ml (10 m.mole) of bromine in 30 ml of glacial acetic acid is added dropwise at room temperature over a period of 30 minutes to 5.25 g (10 m.mole) of 3,5-diiodo-L-thyronine in 70 ml glacial acetic acid. After a further 30 minuts 100 ml of water and sodium metabisulphate are added until the solution becomes colourless.

The pH is adjusted slowly to a value of about 4 with 10% caustic soda solution. The precipitate formed in the system is filtered off under vacuum and washed thoroughly with water. On drying in a desiccator over phosphorous pentoxide, 5.4g (89%) of 3'-bromo-3,5-diiodo-L-thyronine is obtained in the form of a colourless product, m.p. 248°C (decomp.). Further purification of the product can be achieved by recrystallisation or by column chromatography. This yields a final

product which is uniform according to thin layer chromatography or high pressure liquid chromatography.

Example 2

3'-Bromo-5'-(^{125}I)-3-iodo-L-thyronine

0.32 μg of 3'-bromo-3-iodo-L-thyronine in 10 μg of buffer solution (0.5 M phosphate, pH 7.5) is added to 0.5 mCi of sodium iodide (^{125}I, no carrier) in the 10 μl of the same buffer solution. The solutions are mixed thoroughly and 9 μg of chloramin T in 10 μl of the same buffer solution is then added. After 30 sec the reaction is stopped by addition of 24 μg of sodium metabisulphate in 10 μl of water. The product is purified by column chromatography. For this purpose a column (10 cm×1 cm diam) of crosslinked, hydroxypropylated dextran gel (Sephadex LH-20) can be used. A mixture of ethyl acetate/methanol/2N ammonia (20/5/2 parts by volume) can be used as eluant. Fractions of about 0.5 ml are collected with the aid of a fraction collector. The fractions containing the desired product are combined.

Instead of column chromatography, paper or layer chromatography (silica gel) can be used to separate off excess radioactive iodide.

Example 3

3'-Bromo-5'-(^{125}I)-3,5-diiodo-thyroacetic acid

0.4 μg of 3'-bromo-3,5-diiodo-thyroacetic acid in 10 μg of phosphate buffer solution (0.5 M, pH 7.5) is added to 0.5 mCi of sodium iodide (^{125}I, free from carrier) in 10 μl of the same buffer solution. 9 μg of chloramin T in 10 μl of the same buffer solution is then added with thorough mixing. After 30 sec. 25 μg of sodium metabisulphate in 10 μl of water is added to terminate the reaction.

Excess radioactive iodide is separate off by subjecting the reaction mixture to descending paper chromatography on a strip of about 10 cm width. A mixture of n-hexane/tert. amyl alcohol/2N ammonia (1/5/6, by volume) can be used with advantage as developer.

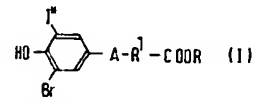
3-bromo-5-(^{125}I)-L-tyrosine

0.3 μg of 3-bromo-L-tyrosine in 10 μl of 0.5 M phosphate buffer solution (pH 7.5) is added to 0.5 mCi of sodium iodide (^{125}I, carrier free) in 10 μl of the same buffer solution. 9 μg of chloramin T in 10 μl of the same buffer solution is added with thorough mixing (e.g. in a vibration mixer). After 30 seconds the reaction is stopped by addition of 25 μg of sodium metabisulphite in 10 μl of water.

Isolation of the tracer is accomplished with advantage by descending paper chromatography with n-butanol/glacial acetic acid/water (4/1/5 parts by volume) as developer. After localisation with the aid of a scanner, the product is eluted. from the appropriate zone with dilute methanolic ammonia.

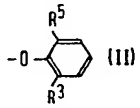
Claims

1. Radioactive carboxylic acid derivatives having the general formula



(I)

where A is a direct bond or a group having the general formula

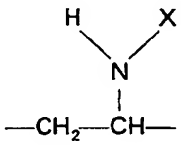


(II)

wherein the ether oxygen is adjacent to the benzene ring shown in formula (I);

R is H or a lower alkyl group;

R^1 is a direct bond, or a $—CH_2—$, $—CH_2—CH_2—$ or



(X=H or a lower alkyl or alkanoyl group); and

R^3 and R^5 may each stand for H, Br or I, independently of each other.

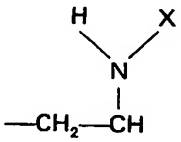
2. Derivatives according to claim 1 wherein A represents a direct bond.

3. Derivatives according to claim 1 wherein A is a group having the general formula (II).

4. Derivatives according to claim 1 wherein R is an hydrogen atom.

5. Derivatives according to claim 1 wherein R^1 is $—CH_2—$.

6. Derivatives according to claim 1 wherein R^1 is the group



7. Derivatives according to claim 6 wherein X is an hydrogen atom.

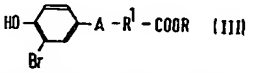
8. Derivatives according to any one of claims 3 to 7 wherein R^3 is an iodine atom.

9. Derivatives according to claim 8 wherein R^5 is also an iodine atom.

10. Derivatives according to any one of the preceding claims wherein the radioactive iodine is ^{125}I or ^{131}I.

11. Derivatives according to any one of the preceding claims wherein they are in the L-, D-, or DL-form.

12. Process for the preparation of the derivatives according to one of the preceding claims, characterised in that a compound having the general formula



(III)

where the symbols have the significance as in claim 1, is reacted in a known manner with a radioactive iodide and with chloramin T.

13. The application of a radioactive carboxylic acid derivative according to any one of claims 1 to 11 in quantitative determination of the concentration of substances in a living organism with the aid of radioimmuno-assay.

14. Radioactive carboxylic acid derivatives according to claim 1 and as described in the examples.

15. A process for the preparation of radioactive carboxylic acid derivatives substantially as herein described with reference to the examples.

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